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The changes in springbok (*Antidorcas marsupialis*) *Longissimus thoracis et lumborum* and *Biceps femoris* muscles during the rigour period

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ABSTRACT

This study describes the changes taking place during rigour in springbok (*Antidorcas marsupialis*) *Longissimus thoracis et lumborum* (LTL) and *Biceps femoris* (BF) muscles. Samples from six male and six female springbok were snap-frozen at 2, 3, 5, 8, 12, 18, 24 and 30 h *post-mortem* (PM) and the pH, calpains I, II and calpastatin activities and cathepsins B, BL and H activities were determined. The temperature was also recorded. Significant third-order interactions were found for the pH and temperature, with the female LTL cooling more rapidly and acidifying slower than the other samples. Female muscles were at risk of developing cold-shortening and all the samples cooled more rapidly than recommended for cattle or sheep. Cathepsin BL activity increased PM, likely due to the degradation of the lysosomes. Calpains I, II and calpastatin activity declined during rigour, indicating that the calpains were activated early PM. Gender and muscle had a significant effect on calpain and cathepsin activity.

1. Introduction

The game industry is one of the most rapid-growing in the agricultural sector in South Africa and contributed ZAR 7.7 billion (ZAR 1 ~ USD 0.12 in 2008) to the economy in 2008 (Thomas, 2012). While the current contribution of meat sales to the total income is very small (Hoffman, 2007), it may in future play a role as not only an additional source of income for farmers but also in improving the food security of the country. It is thus important that the quality of the game meat produced is optimised, and in order to do this the nature of the meat needs to be thoroughly understood. At present springbok (*Antidorcas marsupialis*) are the most important game species for meat production, contributing over 80% of the game carcasses exported in 2005 and 66.5% of the total game harvested by Camdeboo Meat Processors in 2013 (Hoffman & Wiklund, 2006).

The conversion of muscle to meat during the rigour period is a complex process that involves a complete change in the physical and biochemical nature of the muscle (Kim, Warner, & Rosenvold, 2014; Maltin, Balcerzak, Tilley, & Delday, 2003). Two of the most influential and drastic alterations are the decline in the pH and temperature. The temperature of the carcass declines *post-mortem* (PM) as a result of heat dissipation to the surrounding air; with the rate of this decline depending on the size and fat level of the carcass, the metabolic activity of each muscle and the temperature and air flow in the environment (Kim et al., 2014; Lochner, Kauffman, & Marsh, 1980; Smith, Dutson, Hostetler, & Carpenter, 1976). The temperature of the muscle in turn has a large influence on the rate at which any enzymes that still remain active can function, with the enzymes involved in glycolysis being no exception to this. By influencing the activity of the glycolytic enzymes, the temperature can accelerate or retard the conversion of glycogen to lactic acid and thus impact the decline in the pH of the muscle (Jacob & Hopkins, 2014; Newbold & Harris, 1972).

Meat colour, water-holding capacity and tenderness are all highly affected by the nature of the decline in pH and temperature during the rigour period (Hamoen, Vollebregt, & Van der Sman, 2013; Hughes, Oiseth, Purslow, & Warner, 2014; Hwang, Devine, & Hopkins, 2003; Marsh, Lochner, Takahashi, & Kragness, 1981; Savell, Mueller, & Baird, 2005; Thompson et al., 2006). Of these, tenderness has been found to be one of the most important attributes of meat quality for consumers (Bickerstaffe, Bekhit, Robertson, Roberts, & Geesink, 2001), and is influenced by the effect of the changes in pH and temperature on muscle shortening during rigour and the proteolytic activity both during rigour and later during ageing Hwang & Thompson, 2001; (Warriss, 2000).

Two of the proteolytic enzymes thought to play a role in tenderization are the calpains and the cathepsins (Warriss, 2000). The calpains are thought to be activated relatively early PM, and are thus particularly vulnerable to changes in the pH and temperature decline (Dransfield, 1994; Huff Lonergan, Zhang, & Lonergan, 2010). The cathepsins are reported to be active later during conditioning; however, they are contained within the lysosomes in living muscle, and the rate of the decline in pH is thought to influence the rate at which they are released

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into the sarcoplasm (Calkins & Seideman, 1988; Lawrie & Ledward, 2006; O'Halloran, Troy, Buckley, & Reville, 1997). It is therefore important to understand what effect the observed declines in pH and temperature during rigour have on these two enzyme systems.

While studies on the changes during rigour in sheep and cattle have been done, previous research has found that the response of muscle to different rigour temperatures differs between species (Bekhit, Farouk, Cassidy, & Gilbert, 2007; Savell et al., 2005). It is therefore not appropriate to extrapolate findings for beef, lamb or other venison species to springbok meat (Bekhit et al., 2007). Despite this, literature on the nature of the decline in pH and temperature in springbok is extremely limited, and no other research on the changes in proteolytic enzyme activity during rigour in springbok meat has been done.

The purpose of this study was to expand on the current knowledge on pH, temperature and calpain and cathepsin activity changes during the early PM period in springbok meat.

2. Materials and methods

2.1. Harvesting and slaughter

Twelve springbok (six male, six female) were harvested according to standard operating procedure number SU-A CUM13-00,034 at Elandsberg nature reserve near Wellington in the Western Cape of South Africa (33°25′08.0″S 19°01′12.8″E) in January/February of 2014. All the harvested animals were considered mature based on their size, horn growth and tooth eruption. The springbok were either harvested in the early morning before dawn or at night after dark in order to allow the use of a spotlight to locate and temporarily immobilise them, thereby minimising stress. The springbok were killed with a shot to the head from a .308 or .270 calibre rifle and a note was made if the springbok appeared to experienced *ante-mortem* stress. Each springbok was picked up immediately after being shot and the throat was cut to allow for exsanguination. The bled carcasses were transported to the meat processing facility at the Department of Animal Sciences, University of Stellenbosch, where they were skinned and eviscerated within two hours PM. Once dressed, the warm carcasses were placed in a cool room (5.4 ± 0.60 °C) to undergo rigour. All carcasses were suspended by both Achilles tendons in order to ensure equal contraction of the muscles in both sides of the carcass. Bled carcass mass and warm carcass mass were recorded before and after dressing respectively.

Ethical clearance for this study was issued by the Stellenbosch University Animal Care and Use Committee (Ethical clearance number SU-ACUM13-0034).

2.2. Sampling and in situ measurements

The temperature decline of the carcasses, as well as the ambient temperature to which each carcass was exposed, was recorded using automatic temperature loggers (LogTag TREX-8 temperature recorder fitted with a ST100T-15 temperature probe; LogTag, Auckland, New Zealand). These were inserted within two hours PM, with the left *Longissimus thoracis et lumborum* (LTL) being monitored at approximately 2 cm from the spinous processes between the first and second lumbar vertebra and the *Biceps femoris* (BF) at its approximate centre on both the horizontal and vertical planes. The loggers recorded the temperature of the muscle or air every minute for 31 h or until sampling was complete. LogTag Analyser version 2.3 software was used to download the data.

Samples for chemical analysis were taken at eight time periods PM, namely 2, 3, 5, 8, 12, 18, 24 and 30 h. In order to maintain the degree of contraction and the rate of chilling as close to normal as possible the whole muscles were not excised from the carcass. Portions were removed from each muscle at each time period, with four portions being removed from the muscles on each side of the carcass and the position of the portion for each time period being randomly determined. The entire BF was used for sampling whereas the LTL was sampled from between the last lumbar vertebra caudally and the junction with the scapula cranially. As each time period was reached the assigned portion was removed from the carcass and divided into samples for each relevant chemical analysis. The samples were snap-frozen in liquid nitrogen, with the precise time of freezing being recorded. All samples were stored at −80 °C until analysis.

2.3. Chemical analysis

2.3.1. pH

The muscle pH was determined using the sodium-iodoacetate method (Jeacocke, 1977) as described in full by Geldenhuys, Muller, Frylinck, and Hoffman (2015).

2.3.2. Cathepsin activity

The total extractable cathepsin B, B and L (BL) and H activity was determined using enzyme-specific substrates according to the methods of Thomas, Gondoza, Hoffman, Oosthuizen, and Naudé (2004) and Van Jaarsveld, Naude, and Oelofsen (1998). Minor adjustments to the methods were made, with the full methodology being described by Geldenhuys et al. (2015). Cathepsin specific activity was defined as the change in fluorescence measured (excitation 360 nm, emission 460 nm) per minute per mg of extractable protein (Δfluorescence/min/mg protein).

2.3.3. Calpain activity

The activities of the extractable calcium-activated proteases (calpain I and calpain II) and their inhibitor calpastatin were determined according to a combination and modification of the methods described by Dransfield (1996) and Geesink and Koohmaraie (1999). The modifications were made by the Agricultural Research Council's Animal Production Institute (ARC-API), with the methodology being described in full by Geldenhuys et al. (2015). One unit of calpain activity (U) is defined as a 1.0 increase in the absorbance at 366 nm per hour at 25 °C. One unit of calpastatin activity is defined as the amount that inhibited one unit of calpain II activity. Specific activity levels are expressed per mg of extractable protein (U/mg protein).

2.4. Statistical analysis

The trial had a completely randomised design, with the three main effects – gender, muscle and time PM – being tested in a three-factor factorial experiment. Statistical analysis was performed using the statistical software programme Statistica version 12. Initial processing of the data included testing for normality using normal probability plots and testing for homoscedasticity using Levene's test. Once these assumptions had been found to hold true, mixed model repeated measures of analysis of variance (ANOVA's) were performed using the VEPAC (variance estimation, precision and comparison) function in Statistica. In the event that the effect of time PM was significant, Fisher's LSD test was used to determine which of the individual time periods differed significantly from one another. Pearson's correlation coefficients (r) were calculated where appropriate to determine whether significant correlations between variables existed.

In addition to the ANOVA tests, non-linear regressions were fitted to the pH and temperature data, with time PM as the independent variable. The equation for the regression had the following structure:

$$y = c + (a - c)(1 - m)^x$$

In this equation (y) represents the dependant variable (pH or temperature), (x) represents the independent variable (time PM), (c) gives an indication of the minimum y-value obtained, (a) is the intercept of the line with the y-axis and (m) is the decay constant.